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Structural Plasticity Induced by Ketamine in Human Dopaminergic Neurons as Mechanism Relevant for Treatment-Resistant Depression

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Abstract

The mechanisms underlying the antidepressant effects of ketamine in treatment-resistant depression are only partially understood. Reactivation of neural plasticity in prefrontal cortex has been considered critical in mediating the effects of standard antidepressants, but in treatment-resistant depression patients with severe anhedonia, other components of the affected brain circuits, for example, the dopamine system, could be involved. In a recent article in *Molecular Psychiatry*, we showed that ketamine induces neural plasticity in human and mouse dopaminergic neurons. Human dopaminergic neurons were differentiated from inducible pluripotent stem cells for over 60 days. Mimicking the pharmacokinetic exposures occurring in treatment-resistant depression subjects, cultures were incubated with either ketamine at 0.1 and 1 μM for 1 h or with its active metabolite (2R,6R)-hydroxynorketamine at 0.1 and 0.5 μM for up to 6 h. Three days after dosing, we observed a concentration-dependent increase in dendritic arborization and soma size. These effects were mediated by the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor that triggered the pathways of mammalian target of rapamycin and extracellular signal-regulated kinase via the engagement of brain-derived neurotrophic factor signaling, as previously described in rodent prefrontal cortex. Interestingly, we found that neural plasticity induced by ketamine requires functionally intact dopamine D3 receptors. These data are in keeping with our recent observation that plasticity can be induced in human dopaminergic neurons by the D3 receptor-preferential agonist pramipexole, whose effect as augmentation treatment in treatment-resistant depression has been reported. Overall, the evidence of pharmacologic response in human inducible pluripotent stem cell-derived neurons could provide complementary information to those provided by circuit-based imaging when assessing the potential response to a given augmentation treatment.

Keywords

inducible pluripotent stem cells, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, brain-derived neurotrophic factor, mammalian target of rapamycin, extracellular signal-regulated kinase, D3 receptor

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Introduction

Treatment-resistant depression (TRD) is among the most important unmet need in Psychiatry. TRD patients are

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commonly defined as subjects that do not respond to at least two standard selective serotonin reuptake inhibitors (SSRI) or tricyclic antidepressant (TCA) treatments; they represent about 20% to 30% of all patients with major depressive disorder (MDD) diagnosis.[1] Generally, TRD patients become eligible for adjunctive treatments, consisting of antipsychotics, dopaminergic (DA) agonists, behavioral therapies, transcranial magnetic stimulation, vagus nerve stimulation, and electroconvulsive treatment (ECT). In TRD, the efficacy of augmentation treatments is variable, leaving a large percentage of subjects with a partial or unsatisfactory therapeutic response. One hypothesis is that different TRD individuals respond differently to different augmentation therapies depending on which component of the underlying brain circuits is mostly affected. This paradigm requires a characterization of defective circuits from the neurobiological standpoint showing evidences of structural, neurochemical, and cellular response to effective augmentation treatment.

While the understanding of the neuroimaging-defined brain circuits in mood disorders is constantly improving,[2] only the recent introduction of inducible pluripotent stem cell (iPSC)-derived neurons technologies[3,4] is beginning to elucidate the human cellular neurobiology of these brain circuits.

Defective Neural Plasticity as Hallmark for TRD and Chronic Stress

The neuropathology of TRD has been poorly understood until recently. A significant breakthrough came from the clinical evidence that exposure to ketamine[5] produced rapid and persistent antidepressant effects in a significant portion of TRD subjects.[6,7] Its enantiomer S-ketamine, administrated intranasally, showed rapid antidepressant and antisuicidal effects[8] and was just approved by food and drug administration (FDA). Experimental evidences suggest that one ketamine metabolite observed in vivo few hours after ketamine administration, (2 R,6 R)-hydroxynorketamine (HNK),[5] is also involved in the antidepressant effects. All these agents indirectly engage the neurotransmission mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and the brain-derived neurotrophic factor (BDNF)-TrkB signaling, activating the pathway of the mammalian target of rapamycin (mTOR) and of the extracellular signal-regulated kinase (ERK), both critical molecular drivers of neural plasticity.[9,10] Accordingly, ketamine and S-ketamine produced these effects by blocking upstream N-methyl-D-aspartate receptors, while HNK by acting on the glutamatergic synapse. These results are of relevance: they suggest that the triggering of neural plasticity is a common denominator for producing antidepressant effects; they imply that defective neural plasticity is a critical cellular pathogenic mechanism of the depressive episode, and they support a glutamatergic-specific targeting of the glutamate-sensitive component of circuits involved in depression.[11] Defective neural plasticity in frontocortical and hippocampal circuits associated to reduced BDNF levels was observed in rodents following chronic stress.[12] These observations partially parallel those in TRD/MDD patients, including reduced hippocampal volume, hypometabolism in prefrontal cortex, and reduced postmortem levels of BDNF and mTOR.[11,13]

Reversibility of Neural Plasticity by Treatment in Specific Brain Circuits

The evidence of reversibility of the defective status of neural plasticity associated to depression and chronic stress has been considered a biomarker indicative of treatment efficacy. This tenet is reinforced by preclinical studies showing that chronic treatments with SSRIs and TCAs are capable of reverting defective neural plasticity induced by chronic stress in rodent acting via glutamatergic frontocortical circuits.[12,14] In human, neuroimaging evidence supports a normalization of the prefrontal cortex dysfunction in those MDD/TRD subject that responds to any antidepressant treatment.[15,16] Renormalization of hippocampal structural deficit was observed in TRD patients following augmentation therapies such as vagus nerve stimulation [17] or ECT.[18] Reduced DA neurotransmission in ventral striatum, amygdala, and hippocampus associated to anhedonia was observed in MDD/TRD patients and rodents exposed to chronic stress.[19,20] Interestingly, pharmacologic augmentation was observed with aripiprazole and pramipexole, two agents that enhance the functioning of the limbic DA system by targeting the presynaptic DA D2/D3 receptors.[21,22] Pramipexole was also reported to enhance response to TRD patients who did not respond to ECT.[23] Consistent functional engagement of the mesencephalic DA system was also observed with ketamine,[19,24] suggesting a role for this mechanism in its therapeutic antidepressant effects. These findings suggest a possible DA-specific, circuit-based segregation of the pharmacologic effects in TRD patients. However, studies aimed to differentiate TRD patients based on this approach are not available yet, being the direct evidence of plasticity-related pharmacologic effects in human DA neurons only recently obtained.

Human iPSC-Derived Neurons as Translational Model for Defective Brain Circuits of TRD Patients

Since inception, the discipline of Neuropharmacology has developed behavioral and cellular models to study neuroactive drugs with the goal to understand their mechanism of action (MoA). Animal models have been

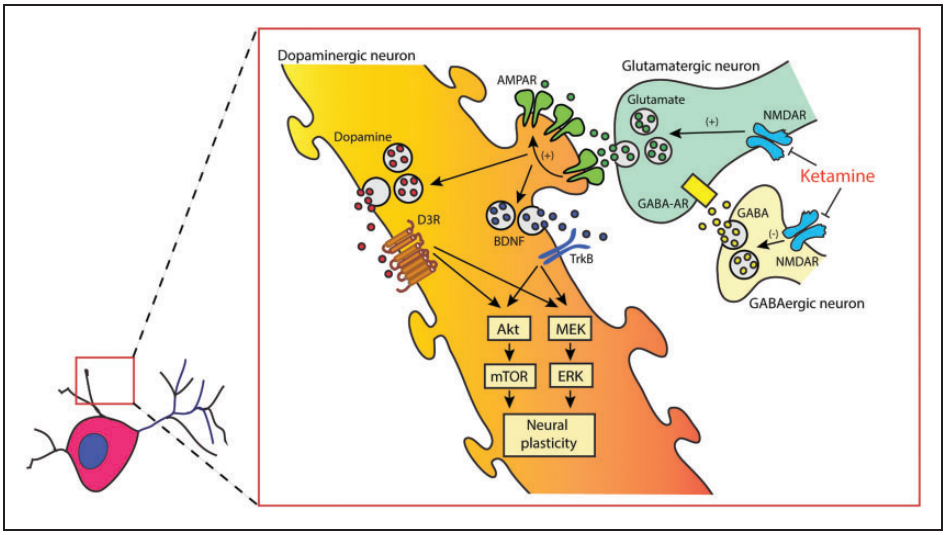

Figure 1. Cartoon representing the putative mechanism of action of ketamine and the molecular signalings involved in determining structural plasticity of dopaminergic neurons in vitro. Akt: thymoma viral proto-oncogene; AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BDNF: brain-derived neurotrophic factor; D3R: dopamine D3 receptor; ERK: extracellular signal-regulated kinase; GABA: γ-aminobutyric acid; GABA-AR: type A γ-aminobutyric acid receptor; MEK: mitogen-activated protein kinase; mTOR: mammalian target of rapamycin; NMDAR: N-methyl-D-aspartate receptor; TrkB: tropomyosin receptor kinase B.

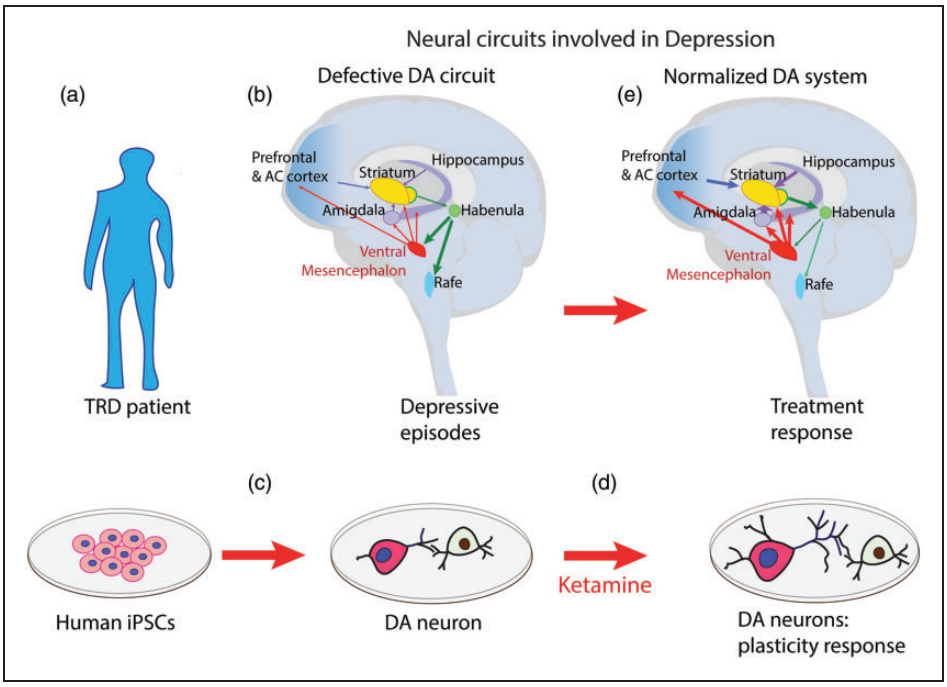

Figure 2. Schematic representation of a proposed translational approach implementing iPSC-derived neurons to assess neural plasticity induced by pharmacological agents potentially active as augmentation antidepressant treatment. In the example (a) a MDD/TRD subject is profiled with neuroimaging for neural circuit involved in depression; (b) a particularly defective DA system is identified; (c) human iPSCs are differentiated to reproduce the neuron phenotype of the circuits involved, in this case DA neurons; (d) iPSC-derived DA neurons are exposed to pharmacologic agents; increase in neural plasticity is obtained with ketamine; (e) ketamine treatment is prescribed, resulting in a neuroimaging and clinical resolution of the episode, with normalization of the state-dependent DA circuit dysfunction. AC: anterior cingulate; DA: dopaminergic; iPSC: inducible pluripotent stem cell; TRD: treatment-resistant depression.

critical for drug development for decades and still they are. However, when used for precise translation into humans, this paradigm may falter. This problem has been particularly relevant for psychiatric disorders, with the failure to translate in effective treatments of several dozens of chemical entities whose preclinical profile was otherwise suggestive of efficacy. Translation failure can be, at least in part, due to subtle but substantial differences in the molecular make-up of human cells versus rodent cells or versus immortalized cell cultures. The recent introduction of human iPSCs has allowed to study in vitro the neurobiology of human neurons with various phenotypes, including the glutamatergic pyramidal neurons of the cortex and the DA neurons of the mesencephalon, adding a new paradigm for the development of novel treatments.[4] Differences and similarities in the MoA of a drug can be now analyzed in standardized cultures of human neurons or organoids.[3,4] As discussed earlier, when assessing the MoA of a pharmacologic agent with putative antidepressant effects, one of the cellular processes expected is neural plasticity.[11]

In a recent article published in *Molecular Psychiatry*,[25] we showed that ketamine and HNK activate plasticity in both human and mouse DA neurons, effects that required an active neurotransmission at the dopamine D3 autoreceptor. We found that ketamine activates BDNF, ERK, and mTOR pathways, showing a molecular signature in DA neurons that overlaps the signature of ketamine in prefrontal cortex.[9,11] Methodologically, we differentiated human DA neurons from iPSCs for 60 to 80 days. The stabilized cultures consisted of a mixture of differentiated DA neurons (30%–35%), GABAergic neurons (20%–25%), and glutamatergic neurons (35%–40%).[25,26] For pharmacologic testing, cultures were exposed to either 1 μM ketamine for 1 h or 0.5 μM HNK for up to 6 h, so to mimic the pharmacokinetic exposures occurring in TRD subjects that received a subanesthetic infusion of ketamine.[26,27] Structural neural plasticity in DA neurons was measured as changes in dendrite and soma morphology three days after exposure, a time considered of relevance in modeling the antidepressant effects of single exposures to ketamine in patients.[6] We observed that structural plasticity at three days was dependent on the activation of both BDNF-TrkB-ERK and PI3K-Akt-mTOR pathways and related to a rapidly induced (within minutes) phosphorylation cascade in the mTOR-p70S6K that persisted for 1 h. These phenomena were driven by an increased AMPAR neurotransmission, as suggested by the blockade of both rapidly induced phosphorylation and structural plasticity produced by pretreatment with the AMPAR antagonists NBQX and GYKI 52466.[25,26] Increased levels of expression of AMPAR subunits GluR1 and GluR2 were observed in the dendrites and soma of DA neurons, respectively, supporting the hypothesis of a ketamine-induced prolonged upregulated AMPAR-dependent function.[28] Immunoneutralization of extracellular BDNF and blockade of TrkB-dependent ERK signaling also prevented ketamine-induced structural plasticity, confirming the critical role of both mTOR and ERK signaling pathways. Finally, we also showed that selective D3R antagonist SB277011-A (but not the D1R antagonist SCH23390) blocked the neural plasticity induced by ketamine and pramipexole, supporting the necessity of a significant autoreceptor-mediated DA tone.[25,26,29] These results are summarized in a cartoon representing the mechanism of structural plasticity in the dendrites of DA neurons (Figure 1). Intriguingly, these findings are suggestive for a possible augmented efficacy of cotreatments with ketamine and pramipexole (or other DA D3R preferential DA agonists); this augmented efficacy could be more important in those TRD subjects with a defective DA component in the limbic circuits involved in depression and, possibly, a relevant symptomatic anhedonia.

Conclusion and Implications

In this commentary, we presented data supporting the possible translational utility of human iPSC-derived DA neurons in modeling the cellular MoA of ketamine, HNK, and pramipexole. Our study suggests that ketamine and HNK effects on neural plasticity are not restricted to the frontocortical and hippocampal component of the brain circuits involved in depression but extends to other circuits, namely, the DA system. Overall, these data together with the recent published data involving the lateral habenula circuit[30] provide further support to the multiplicity of substrates that can be targeted to ameliorate depression in MDD/TRD patients. Advanced neuroimaging will be soon able to cluster subgroups of TRD patients for defective components of the neural circuits involved in depression.[2] This approach, coupled with the in vitro modeling of these circuits using neurons differentiated from hiPSCs (Figure 2), could contribute to the progress toward evidence-based precision treatment.

Authors' Contribution

G.C., E.M.P., and L.C. wrote the manuscript. G.C., E.M.P., and L.C. designed and produced the figures. All authors approved the final version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Emilio Merlo Pich is a full-time employee of Takeda Pharmaceuticals International, Zurich, Switzerland.

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